Thermal Inactivation and Postthermal Treatment Growth during Storage of Multiple *Salmonella* Serotypes in Ground Beef as Affected by Sodium Lactate and Oregano Oil

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ABSTRACT: We assessed the heat resistance of *Salmonella* in raw ground beef in both the absence and presence of sodium lactate, oregano oil, and in combinations of these 2 GRAS-listed ingredients, and determined their bactericidal or bacteriostatic activities during postthermal treatment storage at 15 °C. A cocktail of 8 serotypes of *Salmonella* spp. was inoculated into ground beef supplemented with sodium lactate (NaL) (1.5% and 3%) and/or oregano oil (0.5% and 1%) to obtain approximately 8 log CFU/g. The ground beef samples (3 g) were vacuum-packed and heated at 60, 65, or 71 °C in a circulating water bath for selected times to inactivate approximately 5 to 6 log CFU/g of the pathogen, and then stored at 15 °C for 15 and 30 d. Results show that especially at the lower cooking temperatures, addition of oregano oil increased the inactivation rate of *Salmonella* spp., whereas addition of NaL alone exhibited a protective effect against lethality and decreased the rate. Addition of combinations of oregano oil and NaL overcame this protective effect. During subsequent posttreatment storage for 15 d, *Salmonella* populations in the controls and in samples containing 0.5% oregano (60 and 65 °C) or 1% oregano oil (60 °C) increased to 4.5 to 6 log CFU/g. The values for all other samples were at or near undetectable levels. Results from the 30-d storage study were similar. These findings indicate that lactate and oregano oil may be used to render *Salmonella* spp. more susceptible to the lethal effect of heat and to inhibit growth of *Salmonella* spp. that survive heat treatments.

Keywords: ground beef, oregano oil, *Salmonella*, sodium lactate, thermal inactivation

Introduction

Salmonella are facultatively anaerobic Gram-negative nonspore forming rods belonging to the family *Enterobacteriaceae*. The ubiquitous occurrence of *Salmonella* in the environment, coupled with intensive animal husbandry practices, has favored the prominence of this pathogen in foods and in food ingredients worldwide. The organism has been isolated from 19% to 54% of cattle carcasses, 1.9% of beef samples at retail, and 4.2% of retail chicken samples (Zhao and others 2001; Beach and others 2002).

Salmonella is a leading cause of gastroenteritis in humans. There are about 1.4 million cases of non-typhoid salmonellosis annually in the United States caused by foodborne *Salmonella* serotypes (Crum-Cianflone 2008; CDC 2009). Contaminated ground beef is one of the primary modes of foodborne transmission of this pathogen. Ground beef containing *Salmonella* is a potential health hazard and a continuing concern both for consumers and the foodservice industry (Naugle and others 2006; Phillips and others 2008). One of the important contributing factors that leads to outbreaks of salmonellosis is inadequate time/temperature exposure of food during initial thermal processing (cooking) and during reheating in retail food service establishments or in the home. Therefore, the primary means to control the potential hazard of *Salmonella* in processed meat is effective thermal processing. Accordingly, the U.S. Dept. of Agriculture, in an effort to eradicate this public health hazard, has proposed a 7-D (7-log) reduction in population of *Salmonella* species for cooked beef, ready-to-eat roast beef, and cooked corned beef at point of sale (USDA/FSIS 1999).

The natural antimicrobial, oregano oil, has been reported to inhibit pathogenic bacteria in meat and other foods. Previous studies showed that oregano oil and its major ingredient carvacrol (1) inhibited *Listeria monocytogenes* in meat during storage (Tsigarida and others 2000); (2) was highly active in buffers against both susceptible and antibiotic-resistant foodborne pathogens (Friedman and others 2002, 2004a, 2006; Friedman 2006; Ravishankar and others 2008; Wong and others 2008); (3) inactivated pathogens in apple juice (Friedman and others 2004b), in wine marinades (Friedman and others 2007), and in antimicrobial tomato films (Du and others 2009); (4) inhibited the growth of pathogens in sausages (Busatta and others 2007); (5) inhibited *Clostridium perfringens* spore germination and outgrowth in cooked ground beef (Juneja and others 2006b) and in ground turkey during chilling (Juneja and Friedman 2007); (6) in combinations with cranberry and sodium lactate, inhibited the growth of *L. monocytogenes* in broth and cooked ground beef (Apostolidis and others 2008); and (7) acted synergistically with cranberry against *L. monocytogenes* in fish and meat products (Lin and others 2004).

MS 20090613 Submitted 6/29/2009, Accepted 9/18/2009. *Authors Juneja and Hwang are with Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, U.S.A. Author Friedman is with Produce Safety and Microbiology Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, Albany, CA 94710, U.S.A. Direct inquires to author Juneja (E-mail: vijay.juneja@ars.usda.gov).*

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doi: 10.1111/j.1750-3841.2009.01395.x

Sodium lactate (NaL) has also been reported to exhibit broad antimicrobial effects in meats. Selected previous studies that are relevant to the theme of the present study mentioned in chronological order showed that (1) combinations of lactate and diacetate salts were more effective in inhibiting *L. monocytogenes* and *Salmonella* spp. during storage of beef bologna than either salt alone (Mbandi and Shelef 2002); (2) the heat resistance of *Salmonella* spp. at fixed internal temperatures between 55 and 71 °C increased in the presence of NaL and other salts (Juneja and others 2003); (3) the thermal resistance of *E. coli* O157:H7 was not affected by added NaL (Huang and Juneja 2003); (4) NaL in combination with NaCl extended the quality and shelf life of ground beef (Sallam and Samejima 2004); and (5) a high CO_2 atmosphere did not increase the inhibition of *Salmonella enterica* Typhimurium in ham and pork by NaL or sodium diacetate (Michaelsen and others 2006).

Sodium lactate is currently added to some meat products as a preservative, but has disadvantages. High concentrations of NaL in food may impart an acid taste, add sodium to the diet, and increase resistance of bacteria to thermal inactivation. Previously, we found that certain natural additives, including carvacrol, an isolate of oregano oil, may both decrease growth of *E. coli* and decrease optimal time and temperature combinations required to kill the bacteria in beef (Juneja and Friedman 2008). It is therefore of interest to find out whether the use of lower levels of NaL together with oregano oil will inhibit *Salmonella* in a meat matrix.

The main objective of the present study was to determine heat resistance of *Salmonella* serotypes in raw ground beef and the possibilities of subsequent growth of the pathogen at a temperature abuse of 15 °C. We assessed the ability of NaL and oregano oil, individually and in combination, to reduce the heat resistance of a cocktail of 8 *Salmonella* serotypes in contaminated raw ground beef cooked at 60, 65 and 71 °C, and of subsequent growth of the pathogens at 15 °C. Such information about growth kinetics of injured cells, previously subjected to intervention treatments meant to inactivate the cells, is necessary to model the occurrence and magnitudes of hazards in food that may cause or contribute to adverse outcomes.

Materials and Methods

Materials

Sodium lactate was obtained from Sigma (St. Louis, Mo., U.S.A.) and oregano oil from Lhasa Karnak Herbal Co. (Berkeley, Calif., U.S.A.).

Bacterial serotypes

A cocktail of 8 serotypes of *Salmonella* representing isolates from beef, pork, chicken, turkey, or human clinical cases was used in this study. The information about these serotypes evaluated as a cocktail is given in Table 1. These serotypes were preserved as frozen (−80 °C) stocks in vials containing brain heart infusion broth (BHI; Difco Laboratories Inc., Detroit, Mich., U.S.A.) supplemented with 15% (v/v) glycerol (Sigma Chemical Co., St. Louis, Mo., U.S.A.).

Preparation of inocula. To propagate the cultures, vials were partially thawed at room temperature, and 1.0 mL of the thawed culture was transferred to 50 mL of BHI broth in 250 mL flasks and incubated aerobically for 24 h at 37 °C. Due to the presence of freeze-damaged cells, this culture was not used in heating tests. Two consecutive 24-h transfers were made by using 0.1 mL inocula. These cultures were then maintained in BHI for 2 wk at 4 °C. A new series of cultures was initiated from the frozen stock on a biweekly basis.

Before each experiment, the inocula for conducting the heating studies were prepared by transferring each culture (0.1 mL) to BHI (50 mL) in 250 mL flasks and incubating aerobically for 18 h at 37 °C to provide late stationary phase cells. A primary culture of each serotype was sedimented by centrifuging (5000 × *g*, 15 min, 4 °C) and washed in 0.1% peptone water (PW; wt/vol). This centrifugation and washing step was repeated twice. The cell pellets were finally suspended in PW to a target level of 8 to 9 log CFU/mL. The population densities in each cell suspension were enumerated by spiral plating (Model D; Spiral Biotech, Bethesda, Md., U.S.A) appropriate dilutions (in 0.1% PW), in duplicate, onto tryptic soy agar plates (TSA, Difco, Sparks, Ohio, U.S.A.). The plates were then incubated at 37 °C for 24 h to determine the initial number of bacteria. Approximately equal volumes of each culture were combined in a sterile conical vial to obtain an 8-serotype mixture of *Salmonella* (8 log CFU/mL) before inoculation of meat.

Beef products

Raw 95% lean ground beef, used as the heating menstruum, was obtained from a retail supermarket. The meat was separated into batches for different treatments and mixed thoroughly with NaL (0% to 3%, wt/wt) and/or oregano oil (0% to 1%). The pH of the meats tested were determined using a combination electrode (Sensorex, semi-micro, A.H. Thomas, Philadelphia, Pa., U.S.A.) attached to a pH meter (Orion model 310, Boston, Mass., U.S.A.). The meat was placed into Stomacher 400 polyethylene bags (50 g/bag) and vacuum-sealed. Thereafter, 5 of these bags were vacuum-sealed in barrier pouches (Bell Fibre Products, Columbus, Ga., U.S.A.), frozen at −20 °C and irradiated (25 kGy; 4 h) to eliminate indigenous microflora. Random samples were tested to verify elimination or inactivation of microflora by diluting meat 1 : 1 in 0.1% PW to obtain a slurry, followed by direct surface plating the suspension (0.1 and 1 mL) onto TSA and incubating aerobically at 37 °C for 48 h.

Experimental design

A complete factorial design was used to assess the effects and interactions of heating temperature, NaL, and oregano oil. Levels of the factors studied are as follows: heating temperature: 60, 65, and 71 °C; NaL: 0%, 1.5%, and 3%; oregano oil: 0%, 0.5%, and 1%.

Sample preparation and inoculation

The cocktail of 8 serotypes of *Salmonella* (0.3 mL) was added to thawed, irradiated ground meat (30 g) with added NaL and/or oregano oil. The inoculated meat was blended with a Stomacher 400 Lab-blender (Tekmar, Cincinnati, Ohio, U.S.A.) for 5 min to ensure even distribution of the organisms in the meat sample.

Table 1 — Designations and sources of the 8 *Salmonella* serotypes evaluated in the present study.

Species/serotypes	Original serotype designation	Source	Isolate
Salmonella Thompson	FSIS 120	FSIS	Chicken
Salmonella Enteritidis Phage Type 13A	H3527	CDC	Clinical
Salmonella Enteritidis Phage Type 4	H3502	CDC	Clinical
Salmonella Typhimurium Phage Type DT 104	H3380	CDC	Clinical
Salmonella Hadar	MF 60404	FSIS	Turkey
Salmonella Copenhagen	8457	FSIS	Pork
Salmonella Montevideo	FSIS 051	FSIS	Beef
Salmonella Heidelberg	F5038BG1	CDC	Stuffed ham/ chad slicer

Duplicate ground meat samples (3 g) were then weighed aseptically into 30 × 19 cm sterile filtered Stomacher bags (Spiral Biotech). Thereafter, the bags were compressed into a thin layer (approximately 0.5-1 mm thick) by pressing them against a flat surface, excluding most of the air, and then heat sealed using a Multivac packaging machine (Model A300/16, Multivac Inc., Kansas City, Mo., U.S.A.). Negative controls consisted of bags containing meat samples inoculated with 0.1 mL of 0.1% PW with no bacterial cells.

Thermal inactivation and bacterial enumeration

The thermal inactivation studies were carried out in a temperature controlled circulating water bath (model RTE-17, Digital Plus, NESLAB Instruments Inc., Newington, N.H., U.S.A.) stabilized at 60, 65, or 71 °C as described by Juneja and others (1997). Based on the preliminary studies on heat lethality of about 5 to 6 log CFU/g (data not shown), heating time ranged from 12 min at 60 °C to 30 s at 71 °C. After removal, bags were immediately plunged into an ice–water bath and analyzed within 30 min. Surviving bacteria were enumerated by adding sterile 0.1% PW (3 mL) to each meat sample to obtain a 1 : 1 (w/v) slurry and pummeling for 2 min with a Stomacher 400 lab blender. Decimal serial dilutions of the suspensions were prepared in 0.1% PW and appropriate dilutions, in duplicate, were surface plated onto TSA plates supplemented with 0.6% yeast extract and 1% sodium pyruvate, using a spiral plater.

Samples not inoculated with *Salmonella* cocktail were plated as controls. All plates were incubated at 30 °C for at least 48 h prior to counting colonies. To estimate heat-induced lethality, an average Colony Forming Units per gram value for 4 plates at each sampling point was used for each replicate experiment.

Growth of *Salmonella* during posttthermal treatment

Samples subjected to heating for the duration of the thermal inactivation experiments were quickly cooled and then stored at 15 °C. Population densities were determined at 15 and 30 d as described previously.

Statistical analysis

The heat resistance data were analyzed by analysis of variance (ANOVA) using SAS (2000) to determine if there were statistically significant differences among the treatments. The Bonferroni mean separation test was used to determine significant differences ($P < 0.05$) among means (Miller 1981).

Results and Discussion

Pathogen exposure risk assessment

One of the components of microbial risk assessment is the determination of expected consumption of a microbial pathogen or its toxins (Sheridan and others 1996). The exposure assessment (EA) typically includes assessment of a hazard in a particular food for given scenarios during the production, processing, distribution, and preparation of that food. Thermal processing must typically result in million-fold reductions in bacterial populations in foods. EA analysis also involves estimating the likelihood of pathogens surviving a heat treatment and then growing to highly infectious dose levels that might cause illness if consumed without adequate reheating prior to consumption.

In a relevant study, Juneja and Marks (2006) found that when growth kinetic parameters of *Salmonella* serotypes were assessed following pre- and postthermal (55 °C) inactivation treatment, the lag phase duration times for the postheat treated cells increased at 25 and 37 °C by about 6.2 and 3 h, respectively. Thus, higher growth temperature of cells enhanced the recovery time from injury compared to that occurred at a lower temperature. The 2 chosen temperatures, 25 and 37 °C, represent a region where the growth of *Salmonella* is rapid. While this study provided some characterization of *Salmonella* behavior after thermal treatment, there appears to be a lack of information on the posttreatment growth of *Salmonella* in meat at the temperature abuse conditions encountered during transportation, distribution, and storage and handling in markets, restaurants or by consumers.

Combined effects of sodium lactate and oregano oil on *Salmonella* in ground beef

The present study assessed the effects and interactions of temperature, NaL, and oregano oil on heat inactivation of an 8-serotype *Salmonella* cocktail in 95% lean ground beef with a pH of 5.46. When plating samples, we used TSA supplemented with 0.6% yeast extract and 1% sodium pyruvate for recovery of surviving cells after heating. In preliminary studies, this formulation gave the maximum recovery of heat-injured *Salmonella* compared with recovery on un-supplemented TSA (data not shown).

Cooking beef at 60 °C resulted in 5.7 log CFU/g reduction of *Salmonella* after 12 min of heating (Table 2). However, almost the same level of reduction was achieved within 8 min in beef with added 0.5% or 1% oregano oil. Thus, the pathogen was rendered more sensitive to the lethal effect of heat when beef was supplemented with oregano oil. In contrast, beef with added 1.5% NaL exhibited a protective effect against heat lethality. For example, a 15 min heating time at 60 °C was needed to decrease *Salmonella* cells from 7.92 to 2.23 log CFU/g (5.69 log reduction).

In the present study, NaL did not exhibit a concentration-dependent protective effect towards heat on *Salmonella* in beef because increasing the level of NaL did not result in parallel increase in heat resistance. A 5.33 log CFU/g reduction of *Salmonella* cells was observed at 60 °C in beef with 3% added NaL after 15 min. Nevertheless, the protective effect of NaL presumably arises because added lactate lowered the water activity (a_w) in the meat matrix. For example, Hammer and Wirth (1985) showed a reduction in a_w in cooked liver sausage following the addition of 1% NaL. Similar effects were reported in various model preparations of kamaboco after addition of 7.5% NaL (Kim and Park 1982). However, Jeong and others (1983) reported NaL was effective in lowering a_w of kamaboco when used in combination with other humectants. A related study (Debevere 1989) found that in vacuum-packaged coarse pork liver pate with added 2% NaL, the a_w decreased from 0.959 to 0.945. The effect of reduced a_w on heat resistance is characterized by an increase in protection against the lethal effect of heat. It appears that reducing a_w leads to poor heat penetration through the heating menstruum, thereby accounting for the increased resistance of pathogens in beef.

We found that the undesirable protective effect of NaL in beef was overcome with the addition of oregano oil. The addition of oregano to beef in the presence of NaL increased the heat susceptibility of *Salmonella* (Table 2). For example, while *Salmonella* levels in beef containing 1.5% NaL decreased by 5.69 log CFU/g (from 7.92 to 2.23 log CFU/g) in 15 min at 60 °C, the levels decreased by 6.66 and 6.77 log CFU/g within only 10 min in beef containing 1.5% NaL and 0.5% or 1% oregano, respectively. Supplementation of beef at 60 °C containing 3% NaL with 1% oregano resulted in a reduction of about 6 log CFU/g in 8 min compared to about a 5-log reduction in 15 min. These results show that addition of oregano oil reduces the protective effect of NaL, rendering *Salmonella* in beef more sensitive to heat at 60 °C (Table 2). Similar results were noted at higher temperatures.

Table 2— Heat inactivation of *Salmonella* spp. in ground beef by various treatments at 60, 65, and 71 °C.

Treatment	Initial level[a]	Final level[a]	Log reduction[a]	Inactivation rate[b]
		60 °C		
Control	7.83 ± 0.16	2.14 ± 0.26	5.70 ± 0.11 (12)	0.47 ± 0.01[ab]
0.5% Oregano	7.66 ± 0.03	2.44 ± 0.43	5.23 ± 0.40 (8)	0.65 ± 0.05[ab]
1% Oregano	7.68 ± 0.11	2.54 ± 0.53	5.15 ± 0.64 (8)	0.64 ± 0.08[ab]
1.5% Lactate	7.92 ± 0.19	2.23 ± 0.62	5.69 ± 0.81 (15)	0.38 ± 0.05[b]
1.5% Lactate + 0.5% oregano	8.11 ± 0.53	1.45 ± 0.94	6.66 ± 1.47 (10)	0.67 ± 0.15[ab]
1.5% Lactate + 1% oregano	8.16 ± 0.21	1.39 ± 0.13	6.77 ± 0.33 (10)	0.68 ± 0.03[ab]
3% Lactate	7.82 ± 0.05	2.49 ± 0.05	5.33 ± 0.10 (15)	0.36 ± 0.01[b]
3% Lactate + 0.5% oregano	8.18 ± 0.28	1.16 ± 0.36	7.03 ± 0.08 (12)	0.59 ± 0.01[ab]
3% Lactate + 1% oregano	7.90 ± 0.01	1.94 ± 0.50	5.96 ± 0.51 (8)	0.75 ± 0.06[a]
		65 °C		
Control	7.98 ± 0.16	3.87 ± 0.12	4.11 ± 0.28 (1.5)	2.74 ± 0.28[c]
0.5% Oregano	7.98 ± 0.03	3.56 ± 1.11	4.42 ± 1.14 (1.5)	2.95 ± 1.14[c]
1% Oregano	7.68 ± 0.11	2.29 ± 0.45	5.39 ± 0.34 (2)	2.70 ± 0.17[c]
1.5% Lactate	7.92 ± 0.19	2.50 ± 0.29	5.42 ± 0.48 (1.5)	3.61 ± 0.32[bc]
1.5% Lactate + 0.5% oregano	8.11 ± 0.53	2.84 ± 0.09	5.27 ± 0.62 (0.25)	4.22 ±0.50[abc]
1.5% Lactate + 1% oregano	8.16 ± 0.21	2.11 ± 0.88	6.05 ± 0.68 (1.5)	4.03 ±0.45[abc]
3% Lactate	7.82 ± 0.05	2.87 ± 0.07	4.95 ± 0.12 (2)	2.47 ± 0.06[c]
3% Lactate + 0.5% oregano	7.88 ± 0.28	3.83 ± 0.31	4.05 ± 0.03 (1.5)	2.70 ± 0.02[c]
3% Lactate + 1% oregano	7.88 ± 0.01	3.29 ± 0.24	4.59 ± 0.23 (1.5)	3.06 ± 0.16[c]
		71 °C		
Control	7.83 ± 0.16	2.17 ± 0.59	5.66 ± 0.75 (0.83)	6.82 ± 0.90[b]
0.5% Oregano	7.66 ± 0.03	2.47 ± 0.06	5.19 ± 0.08 (0.83)	6.25 ± 0.10[b]
1% Oregano	7.68 ± 0.11	2.12 ± 0.36	5.57 ± 0.25 (0.83)	6.70 ± 0.30[b]
1.5% Lactate	7.92 ± 0.19	2.23 ± 0.67	5.69 ± 0.86 (1)	5.69 ± 0.86[b]
1.5% Lactate + 0.5% oregano	8.11 ± 0.53	2.66 ± 0.12	5.45 ± 0.65 (0.83)	6.57 ± 0.78[b]
1.5% Lactate + 1% oregano	8.16 ± 0.21	2.68 ± 0.33	5.48 ± 0.13 (0.75)	7.31 ± 0.17[b]
3% Lactate	7.82 ± 0.05	0.97 ± 0.26	6.85 ± 0.31 (0.75)	9.13 ± 0.41[ab]
3% Lactate + 0.5% oregano	8.18 ± 0.28	1.78 ± 1.41	6.40 ± 1.13 (0.5)	12.80 ± 2.26[a]
3% Lactate + 1% oregano	7.90 ± 0.01	1.47 ± 0.30	6.43 ± 0.29 (0.5)	12.85 ± 0.58[a]

[a]Data are means of 2 replicate experiments, each performed in duplicate and expressed as mean ± SD (log CFU/g).
[b]Unit of inactivation = log (CFU/g)/min. Means followed by different letters within a column are significantly different ($P < 0.05$).

Our observations that addition of lactate enhances the survival of *Salmonella* and that other additives may mitigate this effect are consistent with a previous report that sodium pyrophosphate interacted with NaCl (salt), thereby reducing the protective effect of salt on *E. coli* O157:H7 in beef (Juneja and Eblen 1999). These observations support the concept of applying multitarget hurdles to counterbalance the possible negative impact of a single additive.

Inactivation rates of *Salmonella* in ground beef

Inactivation rates of *Salmonella* in ground beef by 8 treatments at 3 temperatures were calculated by dividing the log reductions by the heating time in minutes at each temperature. At 60 or 65 °C, the average inactivation rate (log CFU/min) was not significantly ($P > 0.05$) different in beef with added 0.5% to 1% oregano oil and/or 1.5% to 3% NaL (Table 2). With the exception of beef samples with 3% NaL and 0.5% to 1% oregano oil at 71 °C, there was no apparent reduction in rates of inactivation of the bacteria in the presence of the additives (Table 2). For example, with 3% NaL in beef, the rate of inactivation increased from 6.82 (heated control) to 9.13 log CFU/min, a 33.9% increase by the added lactate. However, this 2.31 log/min increase was not significantly ($P > 0.05$) different from the rate of inactivation in control beef. The corresponding changes were significantly ($P < 0.05$) higher with added 3% lactate and 0.5% oregano oil, as evidenced by a rate of 12.8 log CFU/min, an 87.7% increase. Similarly, with 3% lactate and 1% oregano oil, the reduction rate significantly ($P < 0.05$) increased to 12.85 log CFU/min or an 88.4% increase. These observations indicate that the combination of lactate and oregano oil is more effective in increasing the inactivation rate of the bacteria than are lactate or oregano oil alone. These results also show that the large enhancement in the rates of inactivation of the bacteria with lactate and 0.5% oregano oil was identical to that of lactate and 1% oregano oil.

Table 3— Increase of *Salmonella* spp. population densities during storage (15 °C/15 d) of cooked ground beef mixed without and with added antimicrobials.

	Log CFU/g increase after 15 d of storage[A] (inactivation temperature)		
Treatment	60 °C	65 °C	71 °C
Control	5.97 ± 0.40[a]	5.75 ± 0.44[a]	4.50 ± 0.26[a]
0.5% Oregano	5.35 ± 0.35[a]	4.70 ± 3.21[ab]	ND
1% Oregano	4.55 ± 1.58[a]	ND	ND
1.5% Lactate	0.00 ± 0.00[b]	0.02 ± 0.02[b]	ND
1.5% Lactate + 0.5% oregano	1.05 ± 0.95[b]	ND	ND
1.5% Lactate + 1% oregano	0.55 ± 0.77[b]	0.27 ± 0.37[b]	ND
3% Lactate	ND	ND	0.08 ± 0.11[b]
3% Lactate + 0.5% oregano	ND	ND	ND
3% Lactate + 1% oregano	ND	ND	ND

[A]Data are means of 2 replicate experiments, each performed in duplicate and expressed as mean ± SD. Means followed by different letters within a column are significantly different ($P < 0.05$).
ND = not detected.

Bacterial growth in cooked ground beef during storage for 15 or 30 d without and with antimicrobials

Table 3 compares the increase of bacteria (in log CFU/g) of the ground beef heated at 3 temperatures without and with added antimicrobials. To facilitate understanding the dynamics of

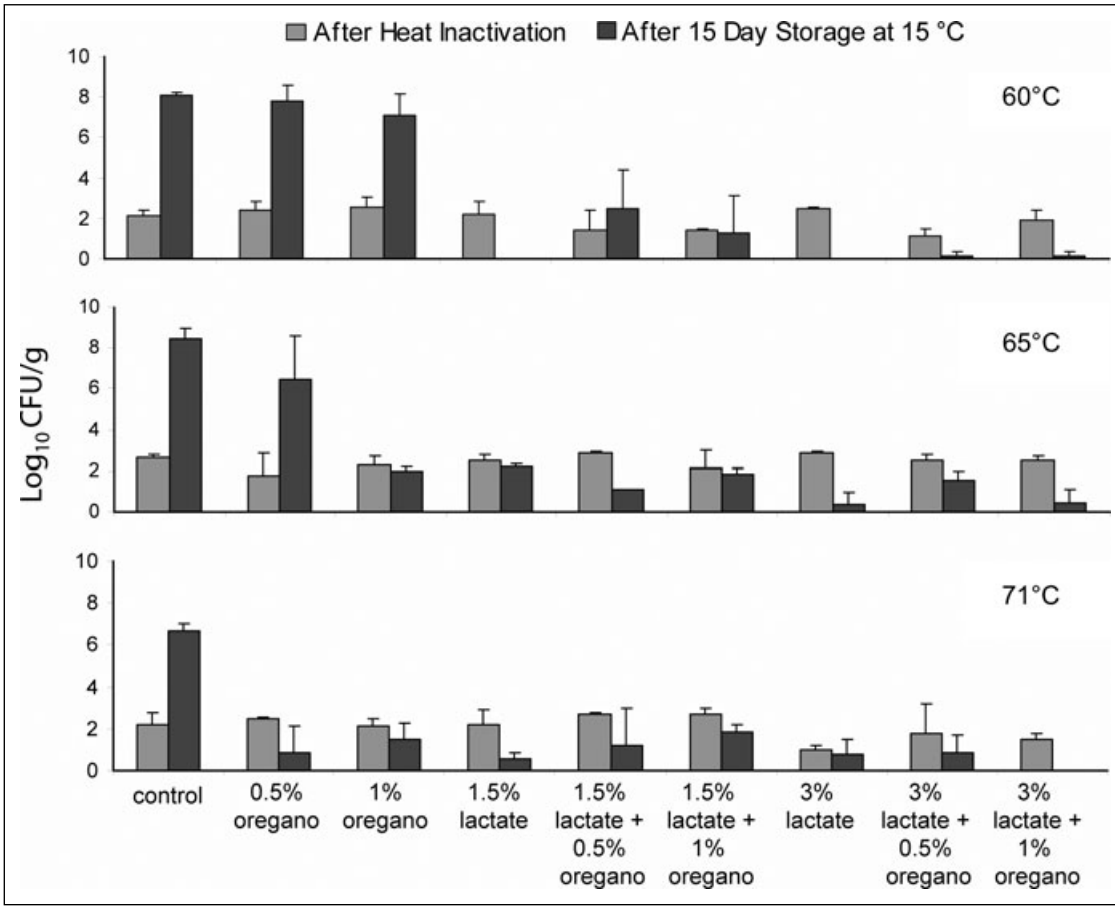

Figure 1 — Bar graphs (mean ± SD) depicting the dynamics of growth and growth inhibition of *Salmonella* spp. in ground beef without and with added antimicrobials (sodium lactate and oregano oil) heated to 3 internal temperatures and stored at 15 °C for 15 d.

inactivation and growth of the bacteria, the observed trends at the 3 temperatures are also depicted as bar graphs in Figure 1. After heating at 60 °C, the increase in *Salmonella* numbers after 15 d of storage at 15 °C ranged from 4.55 to 5.97 $\log_{10}$ CFU/g in beef without or with 0.5% to 1% oregano oil. This increase was significantly higher ($P < 0.05$) than that of the beef samples with added NaL or NaL and oregano oil. While population densities of *Salmonella* in beef without or with 0.5% added oregano heated at 65 °C and stored at 15 °C for 15 d increased by about 5 log CFU/g, the pathogen was either not detected or the level was only 0.27 log CFU/g in beef with added 1% oregano and/or 1.5% to 3% NaL. At a higher heating temperature of 71 °C, surviving cells increased to 4.5 log CFU/g only in heated control samples.

These findings imply that the higher heating temperatures induced a higher level of injury to cells and that the surviving injured cells were sensitive to antimicrobials. The net result in the majority of cases was decreased *Salmonella* population densities during storage to nondetectable levels (Table 3). The cited results show that the added antimicrobials plus heat, but not heat alone, protected the meat samples against growth of *Salmonella* during storage at 15 °C for 15 d. The combination appears to have behaved as bactericidal rather than as bacteriostatic agents.

Table 4 shows the observed growth of *Salmonella* in samples stored at 15 °C for 30 d. The data show that results were similar to results observed at 15 d. With added 0.5% oregano oil, growth took place in samples previously heated at 60 or 65 °C, but not in those heated at 71 °C. With 1% oregano oil, growth occurred in the 60 °C sample, but not in the samples heated at 65 or 71 °C. There was no apparent growth during storage in the samples with added NaL or with NaL and oregano oil.

Table 4 — Growth/no growth of *Salmonella* during storage (15 °C/30 d) of cooked ground beef mixed without and with antimicrobials.

	Growth/no growth *Salmonella* in ground beef previously heated at:		
Treatment (heat only)	**60 °C**	**65 °C**	**71 °C**
Control	+	+	+
0.5% Oregano	+	+	−
1% Oregano	+	−	−
1.5% Lactate	−	−	−
1.5% Lactate + 0.5% oregano	−	−	−
1.5% Lactate + 1% oregano	−	−	−
3% Lactate	−	−	−
3% Lactate + 0.5% oregano	−	−	−
3% Lactate + 1% oregano	−	−	−

The 30-d storage data also imply that exposure of the bacteria in the meat matrix to heat alone appears to induce a bacteriostatic rather than bactericidal effect. Oregano oil alone is bacteriostatic at the lower and bactericidal at the higher temperatures. Lactate alone or in combination with oregano oil was bactericidal at all 3 temperatures.

In a relevant study, Ray (1989) indicated that the assessment of the true lethality of a bacterial population in foods has implications for the microbiological safety of minimally processed foods. This is because the time and temperature employed for the destruction of microbial cells may result in either killing or sublethally injuring the cells. Additionally, the heat-treated cells exhibit a lag period, which may vary from several hours to a few days, before surviving cells begin to grow and multiply.

Calculation of thermotolerance of the cells exposed to heat depends upon the methods used for recovery and enumeration of the surviving heat-treated cells. Microbial cells injured by heat or other environmental stressors may be sensitive to the harsh conditions present in a selective medium and thus unable to resuscitate immediately. Harsh conditions sometimes used to achieve selectivity and suitability for the isolation of uninjured cells may include supplementation of the recovery media with chemicals such as bile salts, NaCl, and deoxycholate. Many injured cells may remain undetected when selective media are used. In the present study, we simulated the conditions that might occur during mild temperature abuse of processed beef and stored samples at 15 °C to allow recovery of the surviving cells after exposure to heat.

Conclusions

For sensory and quality reasons, optimal time and temperature combinations are often not employed when cooking certain ground meat products, such as hamburger patties. Survival of pathogens in undercooked products can result in food poisoning outbreaks. Previously we found that addition of certain natural, food-compatible additives to beef may both decrease microbial growth and decrease optimal time and temperature combinations (Juneja and others 2006a, 2009; Juneja and Friedman 2008).

The results of the present study extend our knowledge about antimicrobial activities of safe food-compatible ingredients in ground beef. Sodium lactate in combination with oregano oil is an effective formulation that can be used to reduce levels of *Salmonella* spp. in ground beef and has the potential to enhance the microbial safety of cooked meat and prevent outgrowth during postthermal storage. Lactate and oregano oil may be used to enhance the sensitivity of *Salmonella* spp. to the lethal effect of heat and to inhibit growth of pathogenic bacteria that survive the heat treatments. These results complement and extend the related observations on the control of *Salmonella* and possibly other foodborne pathogens in ground beef and possibly other foods as well. These findings are expected to assist the retail food industry in designing cooking regimes that ensure the safety of beef contaminated with *Salmonella* serotypes.

Acknowledgment

We thank Carol E. Levin for facilitating preparation of the manuscript.

References

Apostolidis E, Kwon YI, Shetty K. 2008. Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. Int J Food Microbiol 128(2):317–24.

Beach JC, Murano EA, Acuff GR. 2002. Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transport to slaughter. J Food Prot 65(11):1687–93.

Busatta C, Mossi AJ, Rodrigues MRA, Cansian RL, De Oliveira JV. 2007. Evaluation of *Origanum vulgare* essential oil as antimicrobial agent in sausage. Braz J Microbiol 38(4):610–6.

CDC. 2009. Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food–10 States, 2008. MMWR Morb Mortal Wkly Rep 58(13):333–7.

Crum-Cianflone NF. 2008. Salmonellosis and the gastrointestinal tract: more than just peanut butter. Curr Gastroenterol Rep 10(4):424–31.

Debevere JM. 1989. Extended shelf-life from lowered A_w and growth inhibition of lactic acid bacteria by sodium lactate. Fleischwirtschaft 69:223–4.

Du W-X, Olsen CW, Avena-Bustillos RJ, McHugh TH, Levin CE, Mandrell RE, Friedman M. 2009. Antibacterial effects of allspice, garlic, and oregano essential oils in tomato films determined by overlay and vapor-phase methods. J Food Sci 74(7):M390–7.

Friedman M. 2006. Antibiotic activities of plan compounds against non-resistant and antibiotic-resistant foodborne human pathogens. ACS Symp Ser 961:167–83.

Friedman M, Henika PR, Mandrell RE. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. J Food Prot 65(10):1545–60.

Friedman M, Buick R, Elliott CT. 2004a. Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus*. J Food Prot 67:1774–8.

Friedman M, Henika PR, Levin CE, Mandrell RE. 2004b. Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. J Agric Food Chem 52(19):6042–8.

Friedman M, Buick R, Elliott CT. 2006. Antimicrobial activities of plant compounds against antibiotic-resistant *Micrococcus luteus*. Int J Antimicrob Agents 28(2): 156–8.

Friedman M, Henika PR, Levin CE, Mandrell RE. 2007. Recipes for antimicrobial wine marinades against *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*. J Food Sci 72(6):M207–13.

Hammer GF, Wirth F. 1985. Diminution of water activity (A_w) in liver sausage. Proc Eur Meat Res Workers 31:610.

Huang L, Juneja VK. 2003. Thermal inactivation of *Escherichia coli* O157:H7 in ground beef supplemented with sodium lactate. J Food Prot 66(4):664–7.

Jeong HK, Kim DS, Chun SJ, Jo KS, Park YH. 1983. Effect of food humectants on lowering water activity of casing kamaboco. III. Effect of humectant used in combination. Bull Korean Fish Soc 16(2):88.

Juneja VK, Bari ML, Inatsu Y, Kawamoto S, Friedman M. 2009. Thermal destruction of *Escherichia coli O157*:H7 in sous-vide cooked ground beef as affected by tea leaf and apple skin powders. J Food Prot 72(4):860–5.

Juneja VK, Eblen BS. 1999. Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl, and sodium pyrophosphate as controlling factors. J Food Prot 62(9):986–93.

Juneja VK, Marks HM. 2006. Growth kinetics of *Salmonella* spp. pre- and post-thermal treatment. Int J Food Microbiol 109(1–2):54–9.

Juneja VK, Friedman M. 2007. Carvacrol, cinnamaldehyde, oregano oil, and thymol inhibit *Clostridium perfringens* spore germination and outgrowth in ground turkey during chilling. J Food Prot 70:218–22.

Juneja VK, Friedman M. 2008. Carvacrol and cinnamaldehyde facilitate thermal destruction of *Escherichia coli* O157:H7 in raw ground beef. J Food Prot 71(8):1604–11.

Juneja VK, Snyder OP, Marmer BS. 1997. Thermal destruction of *Escherichia coli* O157:H7 in beef and chicken: determination of D- and z-values. Int J Food Microbiol 35(3):231–7.

Juneja VK, Marks HM, Mohr T. 2003. Predictive thermal inactivation model for effects of temperature, sodium lactate, NaCl, and sodium pyrophosphate on *Salmonella* serotypes in ground beef. Appl Environ Microbiol 69(9):5138–56.

Juneja VK, Thippareddi H, Bari L, Inatsu Y, Kawamoto S, Friedman M. 2006a. Chitosan protects cooked ground beef and turkey against *Clostridium perfringens* spores during chilling. J Food Sci 71(6):M236–40.

Juneja VK, Thippareddi H, Friedman M. 2006b. Control of *Clostridium perfringens* in cooked ground beef by carvacrol, cinnamaldehyde, thymol, or oregano oil during chilling. J Food Prot 69(7):1546–51.

Kim DS, Park YH. 1982. Effect of food humectants on lowering water activity of casing kamaboco. II. Effect of lowering water activity of starch, glycine and sodium lactate and prediction of the water activity lowering ability of humectants. Bull Korean Fish Soc 15(1):74–82.

Lin YT, Labbe RG, Shetty K. 2004. Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. Appl Environ Microbiol 70(9):5672–8.

Mbandi E, Shelef LA. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Int J Food Microbiol 76(3):191–8.

Michaelsen AR, Sebranek JG, Dickson JS. 2006. Effects of microbial inhibitors and modified atmosphere packaging on growth of *Listeria monocytogenes* and *Salmonella enterica* Typhimurium and on quality attributes of injected pork chops and sliced cured ham. J Food Prot 69(11):2671–80.

Miller RG, Jr. 1981. Simultaneous statistical inference. 2nd ed. New York: Springer-Verlag. p 67–70.

Naugle AL, Barlow KE, Eblen DR, Teter V, Umholtz R. 2006. U.S. Food Safety and Inspection Service testing for *Salmonella* in selected raw meat and poultry products in the United States, 1998 through 2003: analysis of set results. J Food Prot 69(11):2607–14.

Phillips D, Jordan D, Morris S, Jenson I, Sumner J. 2008. A national survey of the microbiological quality of retail raw meats in Australia. J Food Prot 71(6):1232–6.

Ravishankar S, Zhu L, Law B, Joens L, Friedman M. 2008. Plant-derived compounds inactivate antibiotic-resistant *Campylobacter jejuni* strains. J Food Prot 71(6):1145–9.

Ray B. 1989. Enumeration of injured indicator bacteria from foods. In: Ray B, editor. Injured index and pathogenic bacteria: occurrence and detection in foods, water, and feeds. Boca Raton, Fla.: CRC Press. p 9–54.

Sallam KI, Samejima K. 2004. Microbiological and chemical quality of ground beef treated with sodium lactate and sodium chloride during refrigerated storage. Lebensm Wiss Technol 37(8):865–71.

SAS. 2000. Release 9.1.3. SAS Inst. Inc., Cary, N.C.

Sheridan JJ, Buchanan RL, Montville TJ, editors. 1996. HACCP: an integrated approach to assuring the microbiological safety of meat and poultry. Trumbull, Conn.: Food & Nutrition Press. 198 p.

Tsigarida E, Skandamis P, Nychas GJ. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C. J Appl Microbiol 89(6):901–9.

USDA/FSIS. 1999. Performance standards for the production of certain meat and poultry products. Fed Regist 64(3):732–49.

Wong SYY, Grant IR, Friedman M, Elliott CT, Situ C. 2008. Antibacterial activities of naturally occurring compounds against *Mycobacterium avium* subsp. paratuberculosis. Appl Environ Microbiol 74(19):5986–90.

Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., Area. Appl Environ Microbiol 67(12):5431–6.